6 Problem Formulation for an Assessment of Risk to Honey Bees from Applications of Plant Protection Products to Agricultural Crops

D. Fischer, A. Alix, M. Coulson, P. Delorme, T. Moriarty, J. Pettis, T. Steeger, M. Vaughan, B.E. Vaissière, G. Maynard, M. Kasina, R.C.F Nocelli, C. Scott-Dupree, E. Johansen, C. Brittain, A. Dinter, and J.D. Wisk

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Pesticide Risk Assessment for Pollinators, First Edition. Edited by David Fischer and Thomas Moriarty.

As mentioned in Chapter 5, Phase 1 of the risk assessment process is problem formulation[1] (PF), where measurement endpoints are selected; a conceptual model is prepared that describes a risk hypothesis; and an analysis plan that articulates what data is needed and how it will be used to test the stated hypothesis is described. The PF is intended to provide a foundation for the risk assessment by articulating the purpose of the assessment, defining the nature of the problem (i.e., potential for adverse effects given the nature of the chemical stressor and its existing and/or proposed use), and establishing the plan for analyzing available data and characterizing risk. Participants of the Workshop discussed the generic principles of PF and developed PFs for the assessment of risk of honey bees for two types of pesticide use scenarios: (1) application of a systemic chemical to the soil or seeds planted into the soil, and (2) application of a non-systemic chemical as a foliar spray. It should be noted that there are other possible scenarios such as foliar spray application of a systemic chemical, which may require a separate PF because both contact and oral exposure routes may be important. Likewise, some modification of the PF examples presented herein by the Workshop will likely be needed to apply them to non-*Apis* species in order to account for differences in behavior and life history. The goal here is to illustrate the process for developing a PF for assessment of pesticide risk to honey bees and other insect pollinators by providing some relevant examples.

6.1 WHAT IS PROBLEM FORMULATION?

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PF is the first step of an ecological risk assessment (Figure 6.1). The objective of PF is to develop a working risk hypothesis regarding the potential exposure to and resulting effects of a stressor (e.g., a pesticide) on ecological receptors of concern (e.g., honey bees). During PF, objectives of the anticipated risk assessment are identified and underlying uncertainties and assumptions (constraints) regarding data are described. During PF, initial scoping and integration of available information begins, and data/information gaps are identified. Within the context of a PF for a pesticide risk assessment, the active ingredient is identified as the stressor. To better define the stressor, use information is considered such as: label information, formulations, application parameters (rates, methods, and timing), crop types, or information on target pests (see Text Box).

[1] PF is a widely utilized generic process for framing and developing an ecological risk assessment. This process is not necessarily employed by all regulatory authorities, nor employed in the same manner by those regulatory authorities that do employ the PF process.

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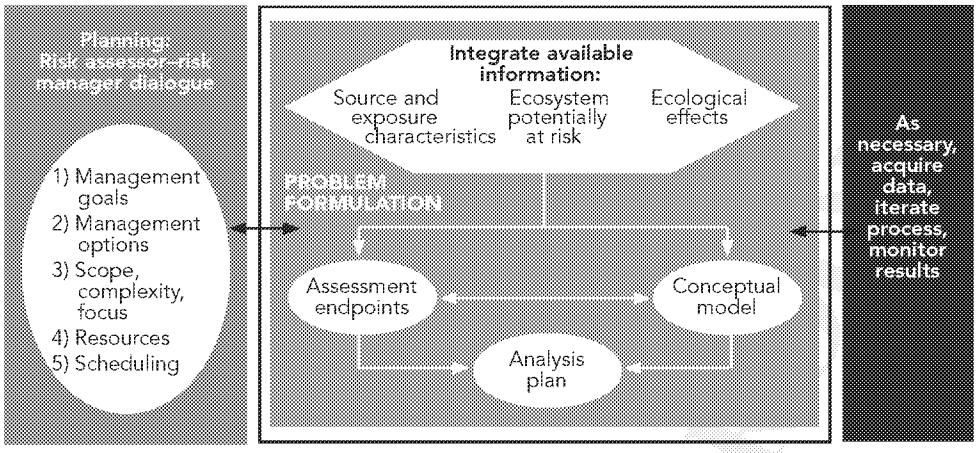

FIGURE 6.1 Scheme depicting problem formulation phase of the ecological risk assessment process. (Taken from USEPA, 1998).

BOX 6.1 PROBLEM FORMULATION QUESTIONS: ASSESSING AVAILABLE INFORMATION

Source and Stressor Characteristics

- What is the source of the stressor (anthropogenic, natural, point source, etc.)?
- What type of stressor is it (chemical, physical, or biological)?
- What is the intensity of the stressor (the dose or concentration, the magnitude, or extent of the disruptions)?
- What is the mode of action? How does the stressor act on organisms or ecosystem functions?

Exposure Characteristics

- With what frequency does the stressor event occur (is it isolated, episodic, continuous)?
- What is the duration of the exposure? How long does it persist in the environment? (half-life, does it bioaccumulate, does it alter habitat, does it reproduce, or proliferate)
- What is the timing of exposure? When does it occur in relation to critical organism life cycles or ecosystem events?
- What is the spatial scale of exposure? Is the extent or influence of the stressor local, regional, global, habitat-specific, or ecosystem-wide?
- What is the distribution? How does the stressor move through the environment?

Ecosystems Potentially at Risk

- In what habitat is the stressor present?
- How do these characteristics influence the susceptibility (sensitivity and likelihood of exposure) of the ecosystem to the stressors?
- Are there unique features that are particularly valued (i.e., the last representative of an ecosystem type)?
- What is the landscape context within which the ecosystem occurs?

- What are the geographic boundaries of the endpoint? How do they relate to the functional characteristics of the ecosystem/endpoint?
- What are the key abiotic factors influencing the endpoint (e.g., climatic, geology, hydrology)?
- Where and how are functional characteristics driving the ecosystem?
- What are the structural characteristics of the ecosystem (e.g., species number and abundance, trophic relationships)?

Ecological Effects

- What are the type and extent of available ecological effects information (e.g., field surveys, laboratory tests, or structure–activity relationships)?
- Given the nature of the stressor (if known), which effects are expected to be elicited by the stressor?
- Under what circumstances will effects occur?

PF has three deliverables (see middle box of Figure 6.1):

1. Risk assessment endpoints that reflect management/protection goals, and the ecosystem they represent;
2. Conceptual models that describe key relationships between a stressor and assessment endpoint; and
3. An analysis plan.

A critical component of PF is planning dialog (left box of Figure 6.1) where risk assessors and risk managers identify and agree on management objectives and identify issues associated with the chemical. PF is intended to be iterative and is informed by existing data (including open literature, existing data, or incident information). As more data become available, the risk hypothesis may change to reflect a more refined understanding of potential risks. The PF identifies available data and information gaps and enables risk managers to convey potential limitations to registrants (chemical manufacturers who support labels) who may be able to provide information to address uncertainties.

Components of PFs include:

1. A description of the nature of the chemical stressor (typically a single technical grade active ingredient, but may include formulations, inerts or degradates of the active ingredient based on the availability of data);
2. A broad overview of pesticide existing/proposed uses;
3. A description of assessment endpoints, that is, valued entities (biological receptors) and their attributes, that is, characteristics to be protected (survival, growth, and reproduction), which are relevant to management/protection goals;
4. A conceptual model that identifies the relationship between ecological entities and the chemical stressor under consideration. The conceptual model has two components, that is, the risk hypothesis and conceptual diagram.
   a. The risk hypothesis describes the predicted relationships among the chemical stressor, exposure and assessment endpoint responses along with a rationale to support the hypothesis.
   b. The conceptual model diagram illustrates the relationships presented in the risk hypothesis and is typically represented by a flow diagram depicting the source (use), stressor, receptor, and change in [endpoint] attribute.

5. An analysis plan is then presented to identify how the risk hypothesis will be assessed; it identifies data needs and methods for conducting the assessment and what measurements, for example, model-estimated environmental concentrations, no-observed-adverse-effect concentrations (NOAEC) and attribute changes, for example, foraging behavior, will be used.

6.1.1 Selecting Assessment Endpoints

Assessment endpoints are explicit expressions of the actual environmental value that is to be protected. Selection of assessment endpoints begins to structure the assessment toward addressing management concerns. Assessment endpoints must be measurable ecosystem characteristics that represent protection goals. Selection of ecological characteristics to protect then becomes the basis for defining assessment endpoints, which connects broad protection goals with specific measures in risk assessment.

The element or characteristic of an ecosystem to be valued or protected must

1. have ecological relevance;
2. be susceptible to known or potential stressors; and
3. be relevant to protection goals and societal values.

6.1.2 Ecological Relevance

Ecologically relevant endpoints reflect important characteristics of the system and may be defined at any level of organization (e.g., individual, community, population, ecosystem, landscape). Ecologically relevant endpoints often help sustain the natural structure, function, and biodiversity of a system or its components.

Ecologically valuable endpoints are those that, when changed, cause multiple or widespread effects (i.e., are upstream of other effects in the ecosystem).

6.1.3 Susceptibility to Known or Potential Stressors

An ecological resource is susceptible when it is sensitive to a stressor, that is, it is affected by the stressor such as through a mode of action. The sensitivity of an ecological resource may be relative to timing, that is, a life stage of an organism (or system), or may be affected by the presence of other stressors or natural disturbances. Measures of sensitivity may include mortality, behavioral abnormalities, loss of offspring, habitat alteration, community structural change, and/or other factors. Susceptibility (of an ecological resource) requires exposure such as through co-occurrence or contact. Typically, the amount and conditions of exposure directly influence how an ecological resource will respond to a stressor. Thus, the timing of exposure, timing of effects, presence or absence of other stressors, and other variables add complexity to evaluations of sensitivity and/or susceptibility.

6.1.4 Defining and Relation of Assessment Endpoints to Protection Goals

As noted earlier, measurement endpoints, assessment endpoints, specific protection goals, and generic protection goals must all be related. Protection goals must be appropriately scaled in order to be represented by assessment endpoints. Assessment endpoints should remain neutral and specific, whereas protection goals represent a desired achievement (i.e., a goal). As such, assessment endpoints do not contain words like "protect," "maintain," or "restore," or indicate a direction for change such as "loss," or "increase." Instead, assessment endpoints are ecological values defined for specific entities and their measurable attributes, providing a framework for measuring stress–response relationships.

Risk assessors and risk managers should share their professional judgment when selecting and defining potential endpoints. Assessment endpoints themselves must be: (i) scientifically valid, (ii) important to the public, and (iii) valued by risk managers (i.e., reflect statutory obligations) in order for them to be relevant. Once ecological values are selected as potential endpoints (attribute changes), they must then be operationally defined. Two elements are required for operational definition:

1. identification of the specific valued ecological entity, such as a species, or a functional group of species, or a community or ecosystem or specific habitat or unique place; and
2. the characteristics (attributes) of the entity that are important to protect.

For practical reasons, it may be helpful to use assessment endpoints that have well-developed test methods, field measurement techniques, and predictive models. However, this is not necessary, as appropriate measures for an assessment endpoint are identified during the development of the conceptual model and further specified in the analysis plan. The number and type of measurement endpoints depend upon the specificity of the questions being asked through the risk assessment and the complexity of the ecological entity being examined. Final assessment endpoint selection is an important risk manager–assessor checkpoint during PF. Risk assessors and risk managers should agree that selected assessment endpoints effectively represent the protection goals.

Common problems in selecting assessment endpoints are:

- the endpoint is a goal
- the endpoint is vague
- the ecological entity is better suited as a measure rather than an endpoint
- the ecological entity may not be sensitive to the stressor
- the ecological entity is irrelevant to the assessment
- the attribute is not sufficiently sensitive for detecting important effects (e.g., survival compared with recruitment for endangered species)

6.1.5 Conceptual Models

Conceptual models provide a written and visual representation of predictive relationships between ecological entities and the stressors and may describe primary, secondary, or tertiary exposure pathways, co-occurrences, ecological effects, or ecological receptors that are reflective of valued attribute changes in these receptors. Multiple conceptual models may be developed to address several issues in a given risk assessment. When conceptual models are used to describe pathways of individual stressors and assessment endpoints and the interaction of multiple and diverse stressors and endpoints, more complex models and sub-models will often be needed.

Conceptual models are flexible and can be modified to accommodate new or additional data. For example, conceptual models can start out as broad and identify as many potential relationships as possible, then narrow as information is acquired. The complexity of a risk hypothesis is commensurate with the complexity of the risk assessment.

Conceptual models consist of two principal components:

1. a set of risk hypotheses that describe predicted relationships among stressor, exposure, and endpoint response; and
2. a diagram that illustrates the relationships presented in the risk hypotheses.

Diagrams are typically flow diagrams with boxes and arrows. Elements considered for inclusion in the diagram include the number of relationships depicted; the comprehensiveness of the information; data abundance or scarcity; or the relative certainty of the pathways. Several smaller diagrams may be more effective than a single diagram that contains too much detail. Diagrams should reflect/document a risk assessor's level of knowledge and degree of certainty regarding its components and should be discussed with risk managers to ensure that they reflect and communicate the manager's concerns prior to analysis.

6.2 CASE 1: PROBLEM FORMULATION FOR A SYSTEMIC CHEMICAL APPLIED TO THE SOIL, OR AS A SEED-DRESSING

6.2.1 Stressor Description

Participants of the Workshop developed a risk assessment process through two case examples that were representative of two general types of pesticide delivery modes, that is, systemic and foliar. Briefly outlined next is an example of a PF for the pesticide risk assessment for pollinators first for a systemic compound, and then for a foliar applied compound.

The stressor of concern is a systemic plant protection product (insecticide or acaricide) applied to the soil of field and orchard crops such as cotton, maize, oil-seed rape, wheat, barley, potatoes, sugar beets, cucurbits (e.g., melons), citrus, and pome fruit, or as a coating on seeds of field crops (cotton, maize, oil-seed rape, wheat, barley). Crop plants absorb the chemical through the roots and translocate it into aboveground tissues of the plant. Magnitude of residue studies demonstrate that the parent compound, *per se*, comprises the residues found in treated plants. Use of the product provides effective control of several economically important chewing and sucking pest insects such as aphids, psyllids, and whiteflies. Application timing is at planting or during transplant of field crops and after flowering of orchard crops.

The above paragraph covers the first two components of a PF, which were listed as (1) a description of the nature of the chemical stressor, and (2) a broad overview of pesticide existing/proposed uses. The third component of a PF is a description of assessment endpoints, that is, valued entities (biological receptors) and their attributes, that is characteristics to be protected (e.g., survival, growth, and reproduction), which are relevant to protection goals.

6.2.2 Protection Goals

As discussed, protection goals are policy decisions that are set by government agencies and other organizations that represent the interests of the societies they serve. In the absence of specific protection goals, the participants used those developed during the Workshop, which included

- protection of pollination services provided by *Apis* and non-*Apis* species;
- protection of honey production and other hive products; and
- protection of pollinator biodiversity.

The first and third of these goals are applicable to pollinators in general (*Apis* and non-*Apis*). The second statement is applicable to managed pollinators (*Apis*).

6.2.3 Assessment Endpoints

For honey bees, logical assessment endpoints are colony strength (population size and demographics) and colony survival (persistence). Bumble bees too can be measured against colony strength (larval ejection,

number of offspring, or colony weight) and colony survival (persistence). As a colony, loss simply represents the situation when colony strength is minimal, it could be argued that *colony survival* is not needed as a separate assessment endpoint. Various measures of colony strength are often made when beehives are rented and placed at agricultural crops. Rental fees are greater for strong colonies than weak colonies because colony strength is expected to be related to the quality of pollination service provided by the colony. Colony strength will likely be significantly impacted if queen viability, brood development, or general worker bee health is adversely affected for an extended period of time. There are many known cases where pesticide exposure has caused effects on colony strength, which meets the criteria for an assessment endpoint which includes:

1. the affected organism has ecological relevance;
2. the affected organism is sensitive, or susceptible to known or potential stressors; and
3. the affected organism is relevant to the management/protection goals and societal values associated with maintenance of pollination services.

For solitary bees, possible assessment endpoints may include adult survival, adult fecundity, larval survival, and larval development time. Populations will be significantly impacted by decreased adult or larval survival and adult fecundity. Increased time for larval development, for example, could impact (be delaying) individual bee emergence time and reduce the number of generations per year in multivoltine species, or cause bees to enter diapauses too late which could ultimately relate to fecundity.

6.2.4 Conceptual Model

The fourth component of PF is the conceptual model that identifies the relationship between ecological entities and the chemical stressor under consideration. The conceptual model has two components: the risk hypothesis and the conceptual diagram.

6.2.4.1 Risk Hypothesis

For a systemic pesticide applied to the soil or as a seed dressing, the risk hypothesis may involve the following steps describing how exposure most likely occurs and results in effects on an assessment endpoint (e.g., colony strength). The hypothesis is:

1. the use of the systemic plant protection product results in concentrations in nectar, pollen, or other parts of plants visited by honey bees;
2. forager honey bees collect the contaminated nectar and pollen and transport it back to the hive where it is incorporated into the food stores of the colony;
3. foragers, hive bees, bee brood, and the queen are exposed to concentrations of the chemical mainly via ingestion;
4. if the exposure concentration is high enough, toxic effects on forager bees, hive bees, bee brood, and/or the queen result in reduced queen fecundity, brood development success, or survival of adult bees; and
5. colony strength is affected as a result of reduced fecundity, brood success, or adult survival.

The duration of exposure of forager bees depends on the persistence of the chemical in the soil and within the treated plants, the duration of bloom, and the chronology of application (planting of treated seeds or

application to the soil) of the chemical to agricultural fields within the landscape around the hive. Based on the risk hypothesis, key questions that need to be answered during risk analysis are:

1. To what extent do foraging honey bees visit treated plants and collect materials (pollen, nectar, resins, honey) that may contain residues of the chemical being assessed?
2. At what level is the parent compound and the toxic metabolite present in materials (pollen, nectar, etc.) collected by honey bees?
3. How do the subject concentrations change over time when stored in the hive?
4. What concentrations in pollen and nectar when fed to a bee colony result in a significant decrease in queen fecundity, brood success, adult survival, and ultimately, colony strength?

6.2.4.2 Conceptual Model Diagram

The conceptual model diagram depicted in Figure 6.2 illustrates the relationships presented in the risk hypothesis for the assessment of risk of a systemic pesticide applied to the soil or as a seed dressing.

The source of exposure is application of the systemic plant protection product to the soil or as a coating to seeds planted in the soil. The primary routes of exposure are assumed to be via residues in pollen and nectar (yellow boxes); however, other routes of exposure such as ingestion of residues in surface water, plant exudates (e.g., guttation fluid), and abraded seed dust are also included. Primary routes of residue transfer are indicated by thick arrows and lesser routes by thin arrows. Forager worker bees may be exposed by both contact and oral ingestion; however, since the chemical is applied to the soil, the potential for contact exposure is assumed to be limited. The attendees of the Workshop believe that the main route of exposure for worker bees is the oral route, particularly the ingestion of nectar, since nectar is the primary food consumed by forager worker bees. Pollen is also collected on hairs on the forager worker bees' bodies, or in small pouches (pollen baskets) on their hind legs. The nectar and pollen collected by worker bees are brought back to the hive where they are incorporated into the food stores, consumed by hive bees, and in turn used to produce food for the queen and the developing brood. If the pesticide concentration is high enough, toxic effects on forager bees, hive bees, bee brood and/or the queen may result in reduced queen fecundity, brood development success, or survival of adult bees. If these effects are severe enough and/or last long enough, a significant effect on colony strength may result.

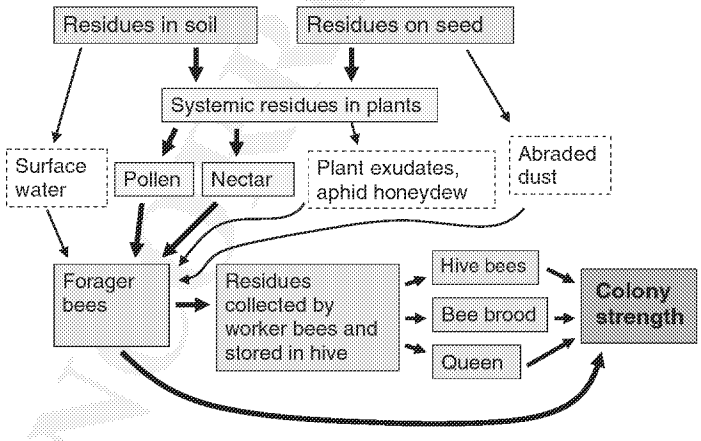

FIGURE 6.2 Depiction of stressor source, potential routes of exposure, receptors and attribute changes for a systemic pesticide applied to the soil or as a seed dressing. (For a color version, see the color plate section.)

6.2.5 Analysis Plan

The final component of the PF is the analysis plan, which identifies how the risk hypothesis will be assessed. The analysis plan identifies the data needs and the methods for conducting the assessment. The analysis plan describes the measures of exposure (e.g., estimated environmental concentrations, monitoring data) and measures of effects (e.g., NOAEC) that will be used. In the case of this example, the analysis plan may generally discuss the attribute changes that will be used for assessing risk to pollinators, including individual bee mortality and colony strength (such as percent coverage of hive frames by adult bees, percent open brood, and/or percent capped brood).

6.2.6 Data Needs for Exposure Characterization

While it may be possible to develop a computer model to predict residues of systemic chemicals in various plant tissues, such models are not currently available and direct measurements are obtained through field studies. For the purposes of this PF, let us assume that field studies have been conducted to measure residue levels of the parent compound and the toxic degradates in pollen and nectar. These measurements can be used to determine the median (50th percentile) and high end (defined here as the 95th percentile) concentrations expected in the pollen and nectar following an application. Estimated daily intake rates for pollen and nectar by various castes of honey bees listed in Table 1 of Rortais et al. (2005) may be used to convert food concentrations (μg chemical/g of food) to a daily dose (μg chemical/individual bee/day). Some toxicity endpoints are expressed in units of a test concentration (e.g., μg chemical/kg test matrix = parts per billion or ppb); or as a dose (e.g., μg chemical/individual bee). The units of the measure of exposure must match the units of the measure of toxicity in order for a valid risk estimate to be calculated.

6.2.7 Data Needs for Effects Characterization

As described briefly in Chapter 8, the progression of effects data development begins with standard laboratory assays and then, if necessary, continues on to higher-tier studies which may consist of specialized laboratory, semi-field and/or field tests. In this sort of testing sequence, the results of higher-tier studies are used to refine the overall conclusions about risk.

Because the main route of exposure expected for systemic chemicals is oral ingestion, toxicity testing of the oral route of exposure is needed to characterize potential effects of residues in bee foods. Standard protocols are available for conducting acute but not chronic oral toxicity tests. Food with residues of systemic compounds may be stored in the hive and used by the colony for long periods of time. The development of a standardized chronic feeding test may be needed. A 10-day feeding test of individual adult honey bees has been proposed by the International Commission on Plant-Bee Relationships (Alix et al., 2009) as a means to provide a chronic toxicity measure. Alternatively, experiments in which whole colonies are fed prescribed concentrations of the test chemical for periods ranging from weeks to months have been performed with some systemic chemicals. Measures of effects of these various chronic tests have included the median lethal concentration and the NOAEC for various colony attributes, including colony strength (e.g., percent frame coverage with adult bees, open brood, or capped brood).

If unacceptable risks cannot be discounted on the basis of simple laboratory test results, and conservative exposure assumptions, then higher-tier studies may be conducted to determine the likelihood and severity of risks under conditions simulating actual agricultural use. Semi-field (tunnel) and field studies may have the advantage of evaluating all routes of exposure simultaneously under conditions reasonably similar to actual field use, whereas laboratory studies are generally limited to evaluation of a single route of exposure under artificial conditions.

6.2.8 Risk Characterization Approach

Most assessments of ecological risks of pesticides use a conventional risk quotient (RQ) or toxicity exposure ratio (TER) approach that compares point estimates of exposure (e.g., typical and high end estimates of residue levels in various food types) to estimated thresholds of toxicity (i.e., median lethal concentration or NOAEC). The RQ equals the exposure point estimate divided by the toxicity point estimate. Although RQ values are dimensionless numbers, the greater the RQ, the greater is the presumed risk. TERs are the reciprocal of the RQ, so the greater the TER, the lower the risk. Regulatory agencies compare the RQ or TER to an established level of concern (LOC) that is presumed to represent a threshold between minimal and non-minimal risk. If the RQ is less than the LOC, or the TER is greater than the LOC, the risk may be presumed to be minimal and further testing is unnecessary provided the constituent elements of the RQ are considered to be sufficiently inclusive. Risk assessment is iterative with screening-level point estimates of exposure and toxicity often used in initial assessments. If the RQ of a screening-level assessment exceeds the LOC, it can be concluded that the risk is potentially not minimal, and further testing may be appropriate to clarify the risk. If semi-field and/or field tests are performed, these results may be incorporated into the risk characterization (provided the studies are of sufficient quality) using a weight-of-evidence approach.

6.3 CASE 2: PROBLEM FORMULATION FOR A CONTACT CHEMICAL APPLIED AS A FOLIAR SPRAY

6.3.1 Stressor Description

The stressor of concern is a "knock-down" insecticide product applied as a spray to field and orchard crops such as cotton, maize, vegetables, citrus, and pome fruit to control pest insects that feed on stems, leaves, inflorescences, and fruit. In this model, the pesticide does not penetrate treated plant surfaces and so it is not translocated systemically throughout the plant (note, however, that certain pesticides that have systemic properties may be foliarly applied). For the purposes of this example, it is assumed that residues on plant foliage dissipate fairly rapidly, with a foliar dissipation half-life of 2–3 days. Because of the short residual toxicity, several applications may be necessary to protect plants during critical phases of the growing season. Based on their chemical structure, none of the chemical's major breakdown products are expected to exhibit significant toxicity to insects. The product label recommends application rates that vary from 20 to 30 g active ingredient (a.i.) per hectare (ha), depending on crop and growth stage.

6.3.2 Management Goals

As discussed earlier, protection goals are policy decisions that are set by government agencies and other organizations that represent the interests of the societies they serve. In the absence of specific protection goals, the participants used those developed during the Workshop, which included

- protection of pollination services provided by *Apis* and non-*Apis* species;
- protection of honey production and other hive products; and
- protection of pollinator biodiversity.

6.3.3 Assessment Endpoints

For honey bees, logical assessment endpoints include colony strength (population size and demographics) and colony survival (persistence). Bumble bees too can be measured against colony strength (larval ejection,

number of offspring, or colony weight) and colony survival (persistence). Since a colony loss simply represents the situation when colony strength is minimal, it could be argued that *colony survival* is not needed as a separate assessment endpoint. Various measures of colony strength are often made when beehives are rented and placed in agricultural crops. Rental fees are greater for strong colonies than weak colonies because colony strength is expected to be related to the quality of pollination service provided by the colony. Colony strength will likely be significantly impacted if queen viability, brood development, or general worker bee health is negatively impacted for an extended period of time. There are many known cases where pesticide exposure has caused effects on colony strength. Colony strength appears to meet very well the identified criteria for an assessment endpoint. Colony strength

1. has ecological relevance;
2. is susceptible to known or potential stressors; and
3. is relevant to protection goals and societal values.

As previously said, for solitary bees, assessment endpoints may include adult survival, adult fecundity, larval survival, and larval development time. Populations will be significantly impacted by decreased adult or larval survival and adult fecundity. Increased time for larval development could impact individual bee emergence time and reduce the number of generations per year in multivoltine species, or by causing bees to enter diapauses too late and, ultimately relate to fecundity and/or a sign that larvae will not emerge as healthy adults. There are known cases where pesticide exposure has affected these endpoints. These endpoints also fulfill the identified criteria for an assessment endpoint (see (1), (2), and (3) above).

6.3.4 Conceptual Model

The fourth component of PF listed previously is the conceptual model, which identifies the relationship between ecological entities and the chemical stressor under consideration. The conceptual model has two components, that is, the risk hypothesis and conceptual diagram.

6.3.4.1 Risk Hypothesis

The risk hypothesis describes the predicted relationships among the chemical stressor, exposure, and assessment endpoint responses along with a rationale to support the hypothesis.

For a nonsystemic pesticide applied as a foliar spray, the risk hypothesis involves the following steps describing how exposure most likely occurs and results in effects on the assessment endpoint (colony strength). The hypothesis is:

1. Residues in spray droplets may (1) contact bees directly (i.e., bees hit directly by the spray), (2) be deposited on plant surfaces visited by honey bees, and (3) contaminate standing water (e.g., puddles) from which bees drink.
2. Spray deposits hitting open flowers may contaminate nectar and pollen sources for a short period of time post-application (until these flowers are replaced by others that were not open during spray).
3. Forager honey bees may ingest contaminated water and/or contaminate nectar, and may collect and transport contaminated nectar and pollen back to the hive where these materials are processed, then incorporated into the food stores of the colony.
4. If the exposure concentration is high enough, toxic effects on forager bees, hive bees, bee brood and/or the queen may result in reduced survival of adult bees, brood development, or queen fecundity.
5. Colony strength is affected as a result of reduced fecundity, brood development, or adult survival if these effects are severe enough or last long enough.

6. As the chemical is knock-down insecticide with short residual time on foliage, the primary effect expected may be direct mortality of forager bees shortly after spraying (i.e., a bee kill event).

The duration of exposure of forager bees will depend on the persistence of the chemical on plant surfaces, and the persistence (duration of bloom) of individual flowers that were hit by the application. As new blooms replace old ones, the potential for exposure may rapidly decrease. Thus, the main concern for foliar spray applications has traditionally been acute exposure of forager worker bees that results in a discrete bee kill event. However the possibility of residues in bee-collected pollen and nectar being brought to, processed and stored in the hive should be considered since this scenario may lead to chronic exposure of the hive bees, queen, and bee brood.

Based on the risk hypothesis, key questions that need to be answered during risk analysis are:

1. To what extent are forager honey bees active when spray applications are made? (or, what is the relation between the application and the flowering of that crop?)
2. If forager bees incur contact exposure during or shortly after application, are the levels of exposure great enough to cause "knock-down" intoxication?
3. If spray deposits represent an initial lethal hazard to honey bees, how long does this situation last?
4. To what extent do foraging honey bees visit sprayed plants and water sources and collect materials (e.g., pollen, nectar, resins, water) that may contain residues of the chemical?
5. What levels of the chemical are present in materials (e.g., pollen, nectar, resins, water) collected by honey bees and brought back to the hive?
6. How do the above concentrations change over time, including changes in concentrations in hive-stored pollen and nectar?
7. What concentrations in pollen, nectar, or beebread when fed to a bee colony result in a significant decrease in queen fecundity, brood development, adult survival, and ultimately, colony strength?

6.3.4.2 Conceptual Model Diagram

The conceptual model diagram depicted in Figure 6.3 illustrates the relationships presented in the risk hypothesis for the assessment of risk of a nonsystemic chemical applied as a foliar spray.

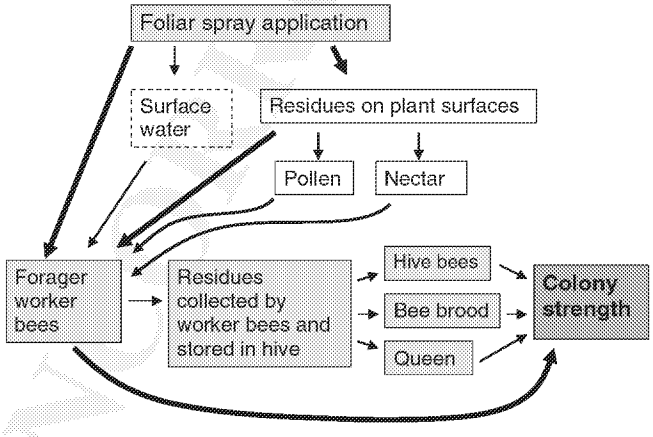

FIGURE 6.3 Depiction of stressor source, potential routes of exposure, receptors and attribute changes for a nonsystemic pesticide applied as a foliar spray. (For a color version, see the color plate section.)

The source of exposure is foliar spray application of the nonsystemic plant protection product to crop plants. The primary routes of exposure are assumed to be via contact of foraging bees with spray as it is applied or with freshly deposited residues on plant surfaces. For flowers open during spraying, residues may occur in pollen and nectar, and these materials may be brought back into the hive, processed and stored as food that is later utilized by hive bees, bee brood, and the queen. Another possible route of exposure is via surface water (e.g., puddles) that are oversprayed and used by bees as a source of drinking water. Primary routes of residue transfer are indicated by thick arrows, lesser routes by thin arrows. Greatest exposure is expected for forager bees that may be exposed via contact with spray droplets and residues on plant surfaces, and via ingestion of residues in water and nectar. If the exposure level is sufficient enough, then forager bees may be killed to the extent that colony strength is reduced (e.g., large bee kill event).

Bees in the hive could also be exposed, but the exposure levels are not expected to be as great as for forager bees unless the hive is inadvertently sprayed (overspray) during application. However, if pesticide residues in the forage area are high, then other bees may be exposed to these high residues during social grooming. In addition, if concentrations in pollen and nectar brought into the hive are high enough, toxic effects on hive bees, bee brood, and/or the queen may result. If these effects are severe enough and/or last long enough, a significant adverse effect on colony strength may result.

6.3.5 Analysis Plan

The final component of the PF is the analysis plan. The analysis plan identifies how the risk hypothesis will be assessed. It identifies data needs and methods for conducting the assessment and what measures of exposure (e.g., estimated environmental concentrations) and measures of effects (e.g., NOAEC) and attribute changes (e.g., colony strength attributes might include estimates of the percent coverage of hive frames by adult bees, open brood, and capped brood) will be used.

6.3.6 Screening Assessment

A simple hazard quotient (HQ) approach is currently used in Europe to predict whether foliar applications of plant protection products have the potential to cause observable bee kills of adult foragers. This screen has been validated by comparing predictions to results of field studies and incident monitoring programs (see Mineau et al., 2008).

The HQ calculation is made as follows:

HQ = application rate (g a.i./ha) / LD50 (µg/bee)
If HQ <50, a minimal risk may be presumed
If HQ >50, a potential risk concern may be presumed (more testing needed)

For example, it is assumed that an acute contact toxicity study has been conducted and the LD50 for the chemical in question is 0.1 µg/bee. Using the maximum application rate of 30 g a.i./ha, the HQ calculation would be 30/0.1 = 300. Since this value is greater than 50, the risk of bee kills cannot be discounted as minimal. Further assessment is needed to evaluate the risk.

6.3.7 Data Needs for Refined Exposure Characterization

A label statement prohibiting application to crops during bloom until the evening or night time hours could go a long way toward eliminating the possibility that foraging bees will be hit by the spray droplets as they

are applied to the crop. A key piece of information needed is how long residues on sprayed vegetation remain toxic to visiting honey bees. This could be estimated from field studies that measure the magnitude and dissipation of residues on sprayed vegetation. It may be simpler to determine this using a standard EPA Tier 2 bioassay, with honey bees i.e., toxicity of residues on foliage (USEPA, 2012) (discussed in greater detail in Chapter 7). Another key piece of information is to determine the residue levels in plant materials (mainly pollen and nectar) collected by forager bees and brought in to the hive. It may be necessary to conduct field studies to obtain direct measurements. Such measurements can be used to determine the median (50th percentile) and high-end (e.g., 95th percentile) concentrations expected to be present in pollen and nectar following an application. Estimated daily intake rates for pollen and nectar by various castes of honey bees listed in Table 1 of Rortais et al. (2005) may be used to convert food concentrations (μg chemical/g of food) to a daily dose (μg chemical/individual bee/day). Some toxicity endpoints are expressed in units of a test concentration (e.g., μg chemical/kg test matrix = ppb); others as a dose (e.g., μg chemical/individual bee). The units of the measure of exposure must match the units of the measure of toxicity in order for a valid risk estimate to be calculated.

6.3.8 Data Needs for Effects Characterization

The logical progression of effects data development is to begin with standard laboratory assays and, if necessary to conduct higher-tier studies that may consist of specialized laboratory, semi-field and/or field tests. In this sort of testing sequence, the results of higher-tier studies are used to refine the assessment and are weighted more heavily in reaching overall conclusions about the risk.

Because the main route of exposure for forager bees is expected to be contact, the standard EPA Tier 2 bioassay with honey bees (i.e., toxicity of residues on foliage (USEPA, 2012) may be appropriate. In this test, groups of honey bees are exposed via contact to vegetation which was sprayed in the field and then collected for testing after prescribed time intervals. For example, a common protocol is to evaluate the contact toxicity of vegetation at 2, 8, and 24 hours post application. In the case of this chemical, let us assume it was found that a high level of mortality occurred in bees exposed to 2-hour-old foliar residues, but that normal honey bee survival was noted when bees were exposed to foliar residues collected 8 and 24 hours after application. Because this is a laboratory-based study, results such as these would indicate that there is a window of acute hazard from contact that exists for 2–8 hours after application of the subject pesticide.

To assess the significance of residues in pollen and nectar that may be brought into and stored in the hive, oral toxicity testing is needed. As a minimum, an acute oral toxicity test can be used to establish oral dose levels that are potentially lethal to adult bees. If there are indications that significant residues will be contained in stored food (pollen, honey, beebread), then a chronic feeding study may be needed to identify the NOAEC. A 10-day feeding test of individual adult honey bees has been proposed by the International Committee on Plant-Bee Relationships (ICPBR) as a means to provide a chronic toxicity measure to adult bees. Various kinds of larval feeding tests have been developed to establish dose levels that affect larval survival and development. Alternatively, experiments in which whole colonies are fed prescribed concentrations of the test chemical for periods ranging from weeks to months have been performed with some chemicals. Measures of effects directly related to colony strength can be obtained from such studies.

If adverse effects cannot be discounted on the basis of simple laboratory test results, higher-tier studies may be conducted to determine the likelihood and severity of effects under conditions simulating actual agricultural use. Semi-field (tunnel) and field studies may have the advantage of evaluating all routes of exposure simultaneously under conditions reasonably similar to actual field use, whereas laboratory studies are generally limited to the evaluation of a single route of exposure under artificial conditions.

6.3.9 Risk Characterization Approach

Calculation of the screening assessment HQ represents an initial risk characterization of the chemical. If the HQ <50, there is a presumption of minimal acute risk in the EU, based on historical investigations of bee kill incidents (Mineau et al., 2008). Based upon the results of the acute toxicity test and the use pattern, higher-tier tests may be required by the EPA, which may provide some insight into whether the label statement requiring applications be made in late afternoon or evening will mitigate the potential risk. Since, in this example, a study showed residual toxicity lasting less than 8 hours, residues from applications made in the late afternoon or evening should not pose an acute hazard to bees that begin foraging the following day. An RQ or TER calculation could be calculated to assess the risk posed by residues in pollen and nectar. The RQ or TER calculation would compare the concentration measured in these matrices or dose taken in by various castes of bees to available toxicity endpoints (LD50, NOAEC, etc.). Finally, well-designed semi-field or field studies may provide the more reliable information regarding the level of risk actually occurring under field use conditions. A weight-of-evidence approach may be taken to integrate the various lines of evidence.

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